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FOREWORD

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E. P. Richards Aug 4, 99

PI - Signature Date

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Introduction

Our aim was to develop a new method for breast cancer diagnosis by measuring prostate specific antigen subfractions in serum.

Body

Please see our appended paper accepted for publication in 'Clinical Cancer Research'.

Key Research Accomplishments

- Diagnostic method devised works
- Sensitivity now limited to 20% of patients
- Specificity is 96%
- Test needs perfection or combination with other markers.

Reportable Outcomes

1. Paper accepted in 'Clinical Cancer Research'

2. M.Sc. obtained by M. Black.

Conclusions

Principle of breast cancer diagnosis with PSA analysis in serum was shown to be feasible. More refinements to improve sensitivity may make the test viable. Clearly, more research is necessary. We are now seeking additional funding from the U.S. Army to supplement this testing with new markers (applications pending approval).

References

See attached Appendix.

Appendices

Manuscript entitled:

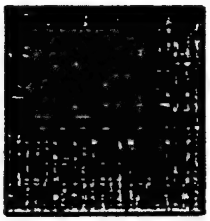
'Serum Total and Free Prostate Specific Antigen for Breast Cancer Diagnosis in Women'

Bindings

Documents are bound as suggested.

Final Reports

Paid employee: M. Black (graduate student).



WAUN KI HONG, M.D.
Deputy Editor

June 11, 1999

Eleftherios P. Diamandis, M.D., Ph.D.
Department of Pathology and Laboratory Medicine
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600 University Avenue
Toronto, Ontario
Canada M5G 1X5

RE: Manuscript # 381115-1

Dear Dr. Diamandis:

Thank you very much for submitting your revised manuscript for publication in *Clinical Cancer Research*. I am very pleased to inform you that this manuscript has been accepted for publication.

Thank you again for submitting this interesting paper. We hope that you will find *Clinical Cancer Research* to be an excellent vehicle for the publication of your work, and we encourage you to submit future manuscripts.

Sincerely,

Waun Ki Hong, M.D.
Deputy Editor
Clinical Cancer Research

WKH:adm

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PATHOLOGY AND LABORATORY MEDICINE
MOUNT SINAI HOSPITAL

Eleftherios P. Diamandis, MD, PhD, FRCPC
Head, Section of Clinical Biochemistry

March 23, 1999
Dr. Waun Ki Hong, M.D.
Deputy Editor, Clinical Cancer Research
Department of Thoracid/Head & Neck Medical Oncology
The University of Texas M.D. Anderson Cancer Center
1515 Holcombe Boulevard
Houston, TX 77030

RE: Manuscript #381115

Dear Dr. Hong,

Enclosed please find four copies of a revised manuscript entitled **Serum Total and Free Prostate Specific Antigen for Breast Cancer Diagnosis in Women** by Margot H. Black, Maurizia Giai, Riccardo Ponzone, Piero Sismondi, He Yu, and Eleftherios P. Diamandis (**Manuscript #381115**). In the manuscript, we reported that free PSA is the predominant molecular form in the serum of breast cancer patients, while PSA complexed to alpha-1-antichymotrypsin is the major molecular form in the serum of women without breast cancer.

The reviewer expressed concern over the usefulness of post-surgical serum PSA measurements, given that only 14% of the breast cancer population relapsed. We feel that this is a very valid point, and have hence refrained from making any postulations with regard to the use of serum PSA for post-surgical monitoring of breast cancer. In addition, a sentence has been added to Page 16 of the Discussion section which indicates that further studies will be needed with larger groups of patients in order to examine the relationship between PSA and individual parameters such as relapse or metastasis.

We hope that the reviewer's concern has been addressed accordingly. Thank you in advance for reconsidering our manuscript for publication.

Sincerely,

E.P. Dia

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Serum Total and Free Prostate Specific Antigen for Breast Cancer Diagnosis in Women[1]

Margot H. Black, Maurizia Giai, Riccardo Ponzone,

Piero Sismondi, He Yu, and Eleftherios P. Diamandis[2]

Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada, M5G 1X5 [M.H.B.,E.P.D]; Department of Gynecologic Oncology, Institute of Obstetrics and Gynecology, University of Turin, 10121 Turin, Italy, [M.G.,R.P.,P.S.]; and Department of Medicine, Louisiana State University Medical Centre, Shreveport, Louisiana, USA, 71130 [H.Y.].

Running Title: Molecular Forms of PSA for Breast Cancer Diagnosis

Key Words: prostate specific antigen, breast cancer diagnosis, benign breast disease, molecular forms of PSA, free/total PSA ratio

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The nonstandard abbreviations used are: ACT, alpha-1-antichymotrypsin; DFP, diflunisal phosphate; Presurg BreastCa, presurgical serum from breast cancer patients; Postsurg BreastCa, postsurgical serum from breast cancer patients.

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ABSTRACT

Prostate specific antigen (PSA) is a serine protease expressed at high levels in prostate epithelium and elevated PSA in serum is a well-established marker of prostate cancer. Recently, the relative proportions of free PSA and PSA complexed to the serine protease inhibitor alpha-1-antichymotrypsin (PSA-ACT) have become important variables in distinguishing between prostate cancer and benign prostatic hyperplasia. Numerous studies have demonstrated the production of PSA in female tissues such as the breast, and low levels of PSA are present in female sera. The objective of this study was to measure and compare the relative proportions of free PSA and PSA-ACT in the serum of women with breast cancer, benign breast disease, or women with no known malignancies. PSA was measured with an established immunoassay for total PSA and a novel immunoassay for free PSA, both with a detection limit of 0.001 μg/L (1 ng/L). The percentage of breast cancer patients with free PSA as the predominant molecular form (>50% of total PSA) in serum was five times higher than that of healthy women or women with benign breast disease, and PSA decreased in the serum of breast cancer patients following surgery. The diagnostic use of free PSA for breast cancer is limited at this point due to the low diagnostic sensitivity (~20%); however, free PSA as the predominant molecular form shows high diagnostic specificity (~96%) in comparison to women free of breast cancer or with benign breast disease. These results suggest that the clinical applicability of free PSA for breast cancer diagnosis and the biological mechanism behind its increase should be further investigated.

INTRODUCTION

Prostate specific antigen (PSA) is a 33kDa single-chain glycoprotein expressed at high levels in the epithelium of the human prostate gland (1). The prostatic function of PSA is to liquefy the sperm-entrapping seminal coagulum following ejaculation (2). The seminal substrates of PSA, a serine protease with chymotrypsin-like specificity, are the major structural constituents of the gel-like coagulum, seminogelin I and II and fibronectin (3).

The name "prostate specific antigen" reflects the initial widespread belief that expression of the protein was restricted to the prostate gland. Over the past five years, however, this notion has clearly been dispelled. Numerous studies have shown that PSA is expressed extraprostatically (4), suggesting that PSA may be functional outside the prostate gland. The periurethral (Skene's) gland was the first female tissue that was suggested to be able to produce PSA (5). This tissue has been referred to as the "female prostate" as its developmental origin is homologous to that of the male prostate (6). It may therefore not be surprising that this gland produces PSA in both males and females. It is now clear that hormonally-regulated tissues in females, such as the breast, can produce PSA. PSA is detectable in normal and hyperplastic breast tissue (7), and is present in the majority of breast tumors (7-10) and breast cysts (11,12). PSA is released into breast secretions, such as the milk of lactating women (13) and nipple aspirate fluid (14-16). Mammary PSA is identical in molecular weight and mRNA sequence to seminal PSA (17). PSA gene expression in breast tumors appears to be under hormonal control as the steroid hormone receptor-positive breast cancer cell lines T-47D and BT-474 can be

induced by androgens, progestins, mineralocorticoids, and glucocorticoids to produce PSA (18,19) *in vitro*.

In males, low levels of PSA are normally released into the blood (20). The majority of circulating PSA occurs in a complex with the serine protease inhibitor alpha-1-antichymotrypsin (ACT) while the remainder (<30%) occurs in the noncomplexed "free" form (21). The use of serum PSA as a prostatic tumor marker has flourished over the past decade. PSA immunoassays are widely used to detect early stage prostate cancer, to evaluate disease progression, and to assess therapeutic response (22). In addition to the total serum PSA level, the ratio of free to total PSA has become an important variable for distinguishing between males with benign and malignant prostatic pathology. The percentage of free serum PSA is lower in males with prostatic carcinoma than in those with benign prostatic hyperplasia or with no apparent prostate pathology (23). While the total PSA level alone is not specific enough for the early diagnosis of prostate cancer, the ratio of free to total PSA may improve specificity without compromising sensitivity (24).

Detectable circulating levels of PSA, likely originating from breast tissue, are present in the serum of women (25). The PSA concentration in female sera is approximately 1000-fold less than that of males (<0.004 μg/L) (26). Given the variation in the ratio of the molecular forms of PSA in prostate cancer, benign prostatic hyperplasia, and normal males, preliminary investigations of the PSA molecular forms in the sera of females were performed in our laboratory. Using chromatographic techniques and a small number of patients, we demonstrated that the predominant form of PSA in the sera of women without breast cancer is PSA bound to ACT, while free PSA constitutes the major form of PSA in the presurgical serum of breast cancer patients (27-30).

The difference in molecular forms of serum PSA between women with and without breast cancer is intriguing. The rationale for this study was that previous studies examining this phenomenon have been performed exclusively with high performance liquid chromatography (HPLC). HPLC can effectively resolve PSA subfractions, but is of limited utility as samples become diluted during the separation procedure. Therefore, to obtain immunologically measurable HPLC fractions, the initial serum total PSA concentration must be at least 0.015 μg/L (15 ng/L) (29). This concentration requirement limits the number of samples that can be analyzed, as less than 10% of female sera have such a relatively high total PSA concentration (29).

Direct immunoassay for free, in addition to total, PSA is a much more efficient method of analyzing large numbers of samples. Such a PSA immunoassay, suitable for analysis of female serum, must have an extremely low detection limit (0.001 μg/L or 1 ng/L). This level of sensitivity is difficult to achieve and, until now, only one such assay for total PSA has been reported (30). An ultrasensitive immunoassay for free PSA with a detection limit of 1 ng/L has recently been developed in our laboratory (31). The use of this assay, in conjunction with our total PSA immunoassay, has allowed us to investigate the molecular forms of PSA in the sera of a large number of women with various pathologies.

MATERIALS AND METHODS

Patient Population. Patients were operated at the Department of Gynecologic Oncology in Turin, Italy. Total and free PSA were measured in 118 serum samples from breast cancer patients and in 115 samples from the same breast cancer patients 6 months following surgery. The sera from 46 patients with breast cysts and 116 patients with uterine fibroids were also analyzed for total and free PSA.

Breast cancer patient age ranged from 30 to 88 years, the median being 56. Each tumor was graded histologically according to the criteria described by Bloom and Richardson (32). The tumor grade distribution was 20% grade 1, 50% grade 2, and 30% grade 3. The tumor size, recorded as the maximum diameter of the fresh surgical specimen, ranged from 0.4 to 8.0 cm with a median of 2.0 cm. 35% of the patients had nodal involvement. 65% and 61% of patients showed estrogen and progesterone receptor positivity, respectively. 14% of the patients had relapsed. 18% of the patients were receiving no adjuvant treatment, 60% were receiving hormonal adjuvant treatment (Tamoxifen), 20% were receiving chemotherapy, and 2% were receiving a combination of chemotherapy and hormonal therapy.

Sera from 99 female blood donors were analyzed for total and free PSA. These samples were collected from the Red Cross Society in Toronto, Canada. The blood donors were confirmed by interview to be free of apparent malignancies and were between the ages of 45 and 65, with a median age of 51. All samples were stored at $-80^{\circ}C$ until analysis.

PSA Analysis. Sandwich-type enzyme-linked immunosorbent assays (ELISAs) were employed for PSA quantitation. Two different pairs of PSA-specific monoclonal antibodies were used for the total and free PSA assay. The free PSA assay demonstrates minimal (<0.5%) cross-reactivity with the PSA-alpha-1-antichymotrypsin complex (PSA-ACT). The total PSA assay measures free PSA and PSA-ACT with equimolarity. Neither assay shows any cross-reactivity with human glandular kallikrein (hK2). The detection limit of both immunoassays is 0.001 μg/L (1 ng/L; this unit will be used throughout the manuscript).

The capture antibody and detection antibody were coded 8301 and 8311 (Diagnostic Systems Laboratories Inc, Webster, TX) for the total PSA assay and F2 and F1 (BiosPacific, Emeryville, CA) for the free PSA assay, respectively. The detection antibodies were biotinylated using Sulfo NHS-LC-LC-Biotin (Pierce, Rockford, IL) at a concentration of 0.2 μg of biotin per μg of antibody. The capture antibody was immobilized on white polystyrene microtitration wells at a concentration of 500 ng/well in 50 mM Tris buffer (pH 7.8). The wells were washed 6 times with wash solution (150 mM NaCl, 50 mM Tris, 1 mM NaN_3, 0.05% Tween 20) after coating overnight. The following incubations at room temperature were employed, followed by washing: (i) 100 μL sera and 50 μL biotinylated detection antibody diluted 1000-fold from a 0.5 mg/mL stock in assay buffer (50 mM Tris, pH 7.8, 10% goat serum, 6% bovine serum albumin, 5% mouse serum, 1% bovine γ-globulin, 0.5% Tween 20, 500 mM KCl) for 1 hour; (ii) alkaline phosphatase-labeled streptavidin (50 ng/mL diluted in 50 mM Tris, pH 7.8, containing 6% bovine serum albumin; Jackson Immunoresearch, West Grove, CA) for 15 min; (iii) diflunisal phosphate (10 mM stock in 10 mM NaOH; prepared in-house) diluted

10-fold in substrate buffer (100 mM Tris pH 9.1, 150 mM NaCl, 1 mM $MgCl_2$, 7.5 mM NaN_3) for 10 minutes. Developing solution (1 M Tris, 0.4 M NaOH, 3 mM EDTA, 2 mM Tb^{3+}), was added to the DFP and the resulting fluorescence was measured on a Cyberfluor 615 Immunoanalyzer (Nordion International, Kanata, ON, Canada) in a time-resolved mode. More details on these time-resolved immunofluorometric procedures can be found elsewhere (30,33).

Statistical Analysis. The Wilcoxon Rank Sum test was used to identify statistically significant differences between the total and free PSA measurements in each patient group. Chi square analysis was used to identify differences in the percentage of patients with detectable total PSA, detectable free PSA, and free PSA as the major serological form (>50% of total PSA). Associations between clinicopathological variables and PSA levels were performed using chi square analysis. Diagnostic sensitivity and specificity were calculated as follows: diagnostic sensitivity = tp/(tp+fn); diagnostic specificity = tn/(tn+fp), where tp = number of true positives, tn = number of true negatives, fp = number of false positives, and fn = number of false negatives. All PSA measurements below the detection limit of the methods (1 ng/L) were adjusted to zero.

RESULTS

Total PSA. The medians and ranges of values for total PSA (free PSA+PSA-ACT) are shown in **Table 1**. The Wilcoxon rank sum test was used to determine any significant differences between the groups. All patient groups had significantly higher total PSA values compared to controls ($p<0.025$). Breast cancer patients had higher total PSA levels before surgery than after surgery ($p=0.047$), and patients with breast cysts had significantly higher levels of total PSA than presurgical breast cancer patients ($p=0.001$).

The percentage of patients in each group with detectable serum total PSA is shown in **Table 2**, and **Figure 1**, with the detection limit being 1 ng/L. The chi square test was used to determine differences between the proportions of patients with detectable total PSA. A higher proportion of patients in every patient group had detectable total PSA compared with the control group ($p<0.015$). A significantly higher percentage of breast cancer patients had detectable total PSA before surgery than after surgery ($p=0.016$). A greater percentage of patients with breast cysts had detectable total serum PSA in comparison to breast cancer patients ($p=0.003$).

Free PSA. The medians and ranges of values for free PSA are also shown in **Table 1.** The Wilcoxon rank sum test was used to determine any significant differences between the groups. Patients with breast cancer (presurgical) had significantly higher free PSA compared to patients without breast disease and controls ($p=0.0001$). Breast cancer patients had higher levels of serum free PSA before surgery than after surgery ($p=0.0001$). Unlike total PSA, there was no significant difference in serum free PSA levels between patients with breast cancer and patients with benign breast disease ($p=0.28$).

The percentage of patients in each group with detectable serum free PSA is shown in **Table 2** and **Figure 1**, with the detection limit being 1 ng/L. The chi square test was used to determine differences between the proportions of patients with detectable free PSA. A significantly greater percentage of patients with breast cancer had detectable free PSA in comparison to breast cancer patients after surgery, uterine fibroid patients, or controls ($p<0.0006$). However, this parameter did not differ significantly between benign and malignant breast disease ($p=0.32$). A greater proportion of breast cancer patients had detectable free PSA before than after surgery ($p<0.0001$).

Free PSA as the Predominant Molecular Form. The percentage of patients in each group with free PSA as the predominant molecular form, which was defined as free PSA >50% of total PSA, is shown in **Table 2** and **Figure 1**. A significant percentage of breast cancer patients have free PSA as the predominant molecular form in serum, in comparison to all other groups ($p<0.02$). Thus, while there is no significant difference between the percentage of breast cancer patients and benign breast disease patients with detectable free PSA ($p=0.32$), the proportion of breast cancer patients with free PSA as the predominant molecular form in serum is significantly higher in comparison to patients with benign breast disease ($p=0.012$). The specificity of this parameter for breast cancer is further illustrated by its decrease in breast cancer patients following surgery ($p<0.0001$).

Correlation of PSA Levels with Clinicopathological Variables. Detectable total serum PSA (>1 ng/L) in breast cancer patients before surgery was significantly associated with younger patient age ($p=0.023$ using chi square analysis with a cutoff of

58 years). Free serum PSA levels and free PSA as the predominant molecular form were not significantly associated with breast cancer patient age.

Serological total PSA was significantly associated with larger breast tumor size ($p=0.027$ using chi-square analysis with a tumor diameter cutoff of 2.0 cm). A similar trend was observed with free PSA levels but the difference did not reach statistical significance ($p=0.097$). We found no association between tumor size and free PSA as the predominant molecular form.

Serum free PSA was significantly associated with higher histological grade in breast cancer patients. A higher proportion of patients with detectable free PSA in serum had tumors classified as grade 3 while patients with no detectable free PSA tended to have tumors classified as grade 1 or 2 ($p=0.014$). No association was found between histological grade and either total PSA or free PSA as the predominant molecular form.

We could not establish any statistically significant association between any form of serum PSA and steroid hormone receptor status, nodal involvement, or relapse.

Diagnostic Specificity and Sensitivity. **Table 3** summarizes the diagnostic sensitivity and specificity of serum total PSA, free PSA, and free PSA as the predominant molecular form for breast cancer. Total PSA has the highest sensitivity for breast cancer, however, it lacks the specificity to distinguish breast cancer patients from those with benign breast disease or with no breast pathology. Free PSA demonstrates higher specificity for breast cancer, but sensitivity is compromised. Although free PSA as the predominant molecular form lacks sensitivity for breast cancer, it is highly specific in differentiating between patients with breast cancer and benign breast disease or with no malignancy.

DISCUSSION

PSA is one of the most valuable serum tumor markers, having been used successfully for diagnosis and postsurgical management of prostate cancer (34). The routine use of PSA immunoassays for the management of prostatic carcinoma has resulted in a marked rise in the number of prostate cancers detected (34). To further improve the effectiveness of distinguishing prostate cancer from benign prostatic hyperplasia, measurement of various molecular forms of PSA has been introduced into clinical practice (35).

The purpose of this study was to measure the relative proportions of the molecular forms of PSA in the serum of women. Sera from women with breast cancer, benign breast disease, uterine fibroids, and women free of malignancies were analyzed for free PSA and PSA-ACT using an established total PSA immunoassay (30) and a novel free PSA assay (31), both with sufficient sensitivity (1 ng/L) for PSA quantitation in a large proportion of female sera. This study has shown that the predominant molecular form of PSA (>50% of total PSA) in a significant proportion of females with breast cancer is free PSA, and free PSA levels decrease in breast cancer patients following surgery. While a significant proportion of women with breast disease (malignant or benign) have detectable serum free PSA, free PSA as the predominant molecular form in serum is highly specific for breast cancer. The vast majority of healthy women, or women with other endocrinopathies, including benign breast disease, have free PSA as the minor molecular form and PSA-ACT as the predominant molecular form.

Although free PSA as the predominant molecular form appears to be unique to breast carcinoma, we observed an increase in total PSA levels in the serum of all patients in

comparison to the control group. We speculate that the slight increase in total PSA in the serum of women with breast cancer, benign breast disease, or uterine fibroids is the result of a disrupted hormonal balance in these women, triggering the aberrant expression of hormone dependent genes such as PSA. The observation that total PSA is only slightly decreased in breast cancer patients following surgery is an indication that a major component of total PSA (in this case PSA-ACT) is not produced by the tumor cells but more likely by normal breast tissue. Alternatively, free PSA decreases more dramatically post-surgery, strongly indicating that this fraction is produced by breast tumors. It has been reported that the majority of breast tumor PSA occurs in the free form (8, 27), while free PSA and PSA-ACT appear to exist in approximately equal proportions in breast cyst fluid (11). It is likely that the tumor is producing free PSA which is incapable of binding to serine protease inhibitors such as ACT, given the large molar excess of serum ACT in comparison to PSA (28). Alternatively, breast tumors may produce an endopeptidase which causes a post-translational modification (internal cleavage) of PSA produced by the breast, thus preventing complex formation with ACT and increasing the proportion of free PSA (28).

It has been established that prostate cancer patients have a greater fraction of PSA complexed to ACT (21), while we report that a significant proportion of breast cancer patients have a higher percentage of free PSA. The mechanism behind this apparent discrepancy is at present unknown, mainly because the differential generation of PSA molecular forms in benign or malignant prostatic or mammary tissue is not yet clear. It must be kept in mind, however, that although breast and prostate cells both produce PSA, the two tissues differ in a multitude of ways. For example, prostatic and mammary tissue

(healthy, hyperplastic, or malignant) respond differently to hormonal proliferation and differentiation signals (e.g. estrogen versus androgen effects).

Some significant associations between serum PSA and clinicopathological variables were observed. Total PSA levels in serum were lower in older women while free PSA levels did not vary with age. The age-independence of free PSA may enhance its applicability as a diagnostic tool. Total PSA levels in serum were associated with larger tumor size, but no significant relationship was established between serum free PSA and tumor size. Serum free PSA was, however, significantly associated with higher histological grade.

Breast cancer is a leading cause of morbidity and mortality in females of developed countries and is the most common malignancy among North American women (36). It is estimated that by the year 2000, 500,000 women worldwide will die from breast cancer (36). Currently, the most effective way to minimize morbidity and mortality from breast cancer is by early diagnosis and administration of therapy (37). It is thus highly desirable to devise new methods of early diagnosis. Mammography is the most sensitive and specific screening modality for breast cancer, however, data presently available do not warrant a universal recommendation for mammography for all women (38). Numerous attempts are presently being made to identify serological markers of breast tumors. Prospective markers include carcinoembryonic antigen, carbohydrate antigen 15.3, tissue polypeptide-specific antigen, and mammary serum antigen (39-41). However, the diagnostic sensitivity of these markers is very limited and does not exceed 32% (39-41). Similarly, the results obtained here indicate that the diagnostic sensitivity of free PSA as the predominant molecular form is approximately 20%. Free PSA as the predominant

form is, however, highly specific for breast cancer in comparison with benign breast disease and normal women. The high degree of specificity suggests that free PSA may have potential clinical applicability either alone or in combination with other markers.

The physiological role of PSA in breast tissue, normal or hyperplastic, remains unknown. As outlined above, before any speculation can be made regarding the role of mammary PSA, it must first be determined if the protease has enzymatic activity. No model examining the enzymatic activity of PSA in breast cancer cells has been developed. It is clear from previous studies that PSA may have a number of potential roles in breast cancer. PSA has been reported to induce uncontrollable proliferation in osteoblast and fibroblast cell lines (42). PSA was also shown to stimulate cell detachment, suggesting a role of PSA in tumor progression or metastasis (43). It has been demonstrated that PSA degrades the extracellular matrix proteins fibronectin and laminin (42). Since the destruction of the epithelial basement membrane facilitates local invasion, this study identified PSA as a potential modulator of matrix degradation.

Furthermore, PSA is an insulin-like growth factor binding protein-3 (IGFBP-3) protease (44). The proteolysis of IGFBP-3 by PSA consequently decreases the affinity of this binding protein for insulin-like growth factor 1 (IGF-1), causing a concomitant elevation in the concentration of serum IGFs (45), which are known mitogens for breast cancer cells. Furthermore, it has been proposed that IGFBP-3 may be able to interact with breast cancer cells in a manner that is independent of IGFs through specific receptors for the binding protein (46). Antiproliferative effects of IGFBP-3 on several breast cancer cell lines have been reported (47) and it has been suggested that IGFBP-3

may induce apoptosis in breast cancer cells (48). The ramifications of IGFBP-3 degradation on tumor progression are therefore numerous.

In summary, we have demonstrated that free PSA is the predominant molecular form of serological PSA in a significant proportion of breast cancer patients and that free PSA levels decrease significantly in the serum of women with breast cancer following surgery. Although free PSA as the predominant molecular form is highly specific for breast cancer, its clinical utility is limited at this time due to low sensitivity. Given the heterogeneity of the population of breast cancer patients, further studies with larger cohorts of patients are required for the examination of molecular forms of PSA with regard to individual parameters such as risk of relapse or metastasis. Finally, the physiological mechanism behind the free PSA increase in breast cancer and its ramifications with respect to tumor progression should be further investigated.

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Table 1. *Total and Free PSA Levels in Serum of Patients*

Medians and ranges of values for total PSA (free PSA+PSA-ACT) and free PSA in the sera of patient groups and controls.

	TOTAL PSA (ng/L)		**FREE PSA (ng/L)**	
Patient Group	**Median**	**Range**	**Median**	**Range**
Controls (n=99)	0	0-55	0	0-6
Presurg BreastCa (n=118)	1.3	0-8153	0	0-2451
Postsurg BreastCa (n=115)	0	0-359	0	0-22
Breast Cysts (n=46)	3.5	0-221	0	0-25
Uterine Fibroids (n=107)	1.0	0-81	0	0-17
	Statistically Significant Differences Between Groups			
Controls *vs.*				
Presurg BreastCa	**p=0.0001***		**p=0.0001***	
Postsurg BreastCa	**p=0.02***		p=0.81	
Breast Cysts	**p=0.0001***		**p=0.01***	
Uterine Fibroids	**p=0.0045***		p=0.19	
Presurg BreastCa *vs.* Postsurg BreastCa	**p=0.047***		**p=0.0001***	
Presurg BreastCa *vs.* Breast Cysts	**p=0.001***		p=0.28	

*statistically significant difference (Wilcoxon Rank Sum Test)

Table 2. *Detectable Total and Free PSA in Serum of Patients*[a]

The percentage of patients with detectable total PSA (free PSA+PSA-ACT), detectable free PSA, and free PSA as the predominant molecular form (>50% of total PSA) in serum.

PATIENT GROUP	Patients With Detectable Total PSA (%) [a]	Patients With Detectable Free PSA (%) [a]	Patients With >50% Free PSA (%)
Controls (n=99)	28 (29)	10 (10)	3 (3)
Presurg BreastCa (n=118)	67 (57)	37 (36)	24 (20)
Postsurg BreastCa (n=115)	51 (44)	10 (11)	4 (3)
Breast Cysts (n=46)	36 (75)	12 (28)	2 (4)
Uterine Fibroids (n=107)	56 (50)	11 (16)	6 (6)
	Statistically Significant Differences Between Groups		
Controls *vs.*			
Presurg BreastCa	**p<0.0001***	**p<0.0001***	**p=0.0001***
Postsurg BreastCa	**p=0.012***	p=0.79	p=0.85
Breast Cysts	**p<0.0001***	**p=0.007***	p=0.63
Uterine Fibroids	**p=0.012***	p=0.20	p=0.37
Presurg. BreastCa *vs.* Postsurg. BreastCa	**p=0.016***	**p<0.0001***	**P<0.0001***
Presurg BreastCa *vs.* Breast Cysts	**p=0.003***	p=0.32	**p=0.012***

[a] PSA≥1ng/L is considered detectable
*statistically significant difference (Chi Square Test)

Table 3. *Diagnostic Specificity and Sensitivity of PSA*

Diagnostic sensitivity and specificity of serum total PSA (free PSA+PSA-ACT), free PSA, and free PSA as the predominant molecular form (>50% of total PSA).

PARAMETER	DIAGNOSTIC SENSITIVITY (%)[a]	DIAGNOSTIC SPECIFICITY (%)[a]
Total PSA		
Presurg BreastCa vs. Controls	57	69
Presurg BreastCa vs. Breast Cysts	57	25
Free PSA		
Presurg BreastCa vs. Controls	36	90
Presurg BreastCa vs. Breast Cysts	36	72
>50% Free PSA		
Presurg BreastCa vs. Controls	20	97
Presurg BreastCa vs. Breast Cysts	20	96

[a] Diagnostic sensitivity and specificity calculations are described in the *Materials and Methods* section

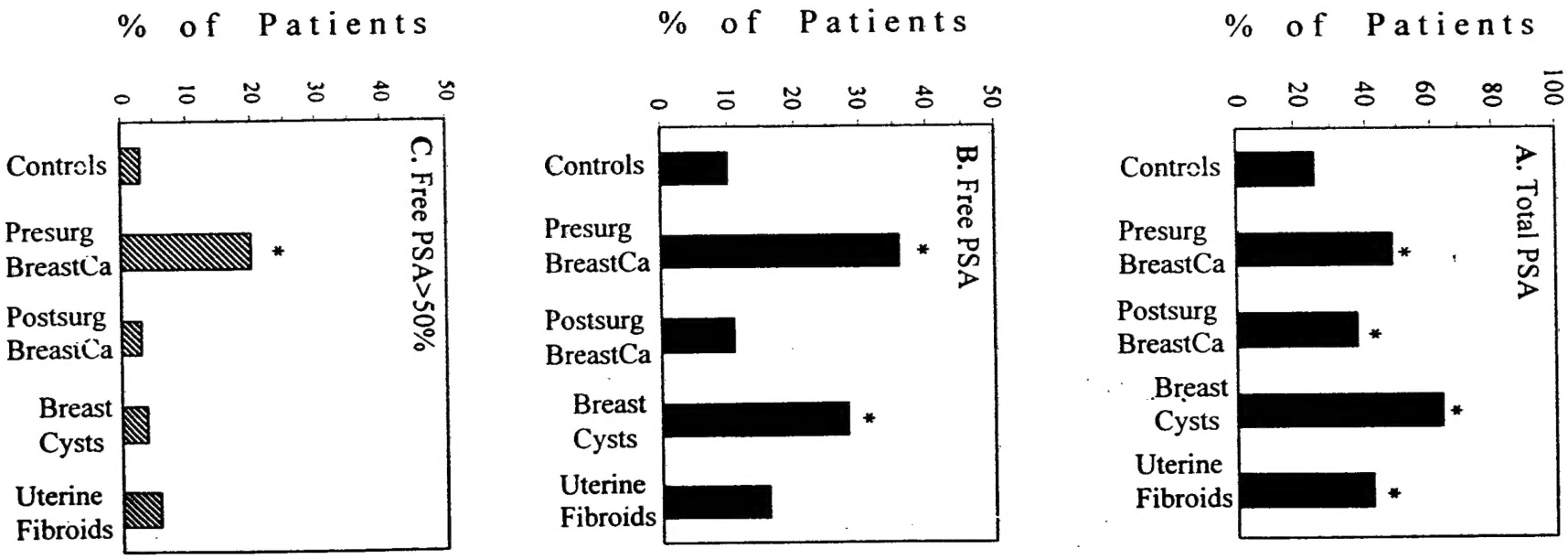

FIGURE LEGEND

Figure 1.
Percentage of patients in each group with (A) detectable total PSA in serum; (B) detectable free PSA in serum; and (C) free PSA as the predominant molecular form. Detectable PSA means PSA≥1ng/L.
* Indicates patient groups which are statistically different from controls (Chi Square Test)